

Pseudo-Streaming Potentials in *Necturus* Gallbladder Epithelium

II. The Mechanism Is a Junctional Diffusion Potential

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ABSTRACT The mechanisms of apparent streaming potentials elicited across *Necturus* gallbladder epithelium by addition or removal of sucrose from the apical bathing solution were studied by assessing the time courses of: (a) the change in transepithelial voltage (V_{ms}), (b) the change in osmolality at the cell surface (estimated with a tetrabutylammonium [TBA⁺]-selective microelectrode, using TBA⁺ as a tracer for sucrose), and (c) the change in cell impermeant solute concentration ($[TMA^+]_i$, measured with an intracellular double-barrel TMA⁺-selective microelectrode after loading the cells with TMA⁺ by transient permeabilization with nystatin). For both sucrose addition and removal, the time courses of V_{ms} were the same as the time courses of the voltage signals produced by $[TMA^+]_i$, while the time courses of the voltage signals produced by $[TBA^+]_o$ were much faster. These results suggest that the apparent streaming potentials are caused by changes of $[NaCl]$ in the lateral intercellular spaces, whose time course reflects the changes in cell water volume (and osmolality) elicited by the alterations in apical solution osmolality. Changes in cell osmolality are slow relative to those of the apical solution osmolality, whereas lateral space osmolality follows cell osmolality rapidly, due to the large surface area of lateral membranes and the small volume of the spaces. Analysis of a simple mathematical model of the epithelium yields an apical membrane L_p in good agreement with previous measurements and suggests that elevations of the apical solution osmolality elicit rapid reductions in junctional ionic selectivity, also in good agreement with experimental determinations. Elevations in apical solution $[NaCl]$ cause biphasic transepithelial voltage changes: a rapid negative V_{ms} change of similar time course to that of a Na^+/TBA^+ bi-ionic potential and a slow positive V_{ms} change of similar time course to that of the sucrose-induced apparent streaming potential. We conclude that the V_{ms} changes elicited by addition of impermeant

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solute to the apical bathing solution are pseudo-streaming potentials, i.e., junctional diffusion potentials caused by salt concentration changes in the lateral intercellular spaces secondary to osmotic water flow from the cells to the apical bathing solution and from the lateral intercellular spaces to the cells. Our results do not support the notion of junctional solute-solvent coupling during transepithelial osmotic water flow.

INTRODUCTION

In the preceding article (Reuss, Simon, and Xi, 1992) we presented experiments which demonstrate that apparent streaming potentials can be elicited in *Necturus* gallbladder epithelium by changing the concentration of an impermeant nonelectrolyte (sucrose) in the apical bathing solution. The results indicate that the "permeant" species in NaCl-Ringer's solution is Na^+ , in agreement with previous studies of paracellular ion permselectivity of this epithelium (Reuss and Finn, 1975; Van Os and Slegers, 1975). Further, circuit analysis based on intracellular-microelectrode data showed that the transepithelial voltage change is of paracellular origin. Finally, an observation was made that suggests a mechanism for the observed phenomenon, namely, the time course of the transepithelial voltage change upon changing the osmolality of the apical bathing solution. Bi-ionic potentials and apparent streaming potentials were elicited in the same preparations under experimental conditions designed to assure similar time courses of the changes in salt and/or nonelectrolyte concentrations at the epithelial surface and the time courses of the transepithelial voltage changes were compared. The result was a 2.5- to 4-fold difference in half-times for the voltage changes, the apparent streaming potential being slower than the bi-ionic potential. This observation suggests that the observed transepithelial voltage change in response to the change in apical solution osmolality is in fact a pseudo-streaming potential (Barry and Diamond, 1984). Specifically, we argue that the change in apical solution osmolality causes water flow from cell to lumen, which elevates the cellular osmolality, in turn causing water flow from the lateral intercellular spaces into the cell. The resulting change in $[\text{NaCl}]$ in the spaces is the immediate cause for the change in transepithelial voltage. In this paper we test this hypothesis by taking advantage of electrophysiologic techniques to compare the time courses of solute concentrations at the three sites of interest, namely, the surface of the epithelium, the cell interior, and the lateral space (the latter indirectly, via the time course of the change in transepithelial voltage). In addition, we analyze the data in terms of a simplified mathematical model of the epithelium. The results indicate that the phenomenon under study is a pseudo-streaming potential. Further, they support the view that because of the high osmotic water permeability and large surface area of the basolateral membrane, and the small volume of the lateral intercellular space, the latter achieves rapidly osmotic equilibrium with the cells. Our results and analysis indicate that the mere observation of apparent streaming potentials across leaky epithelia is insufficient proof of solute-solvent flux coupling in a common transport pathway and hence cannot be used as an argument for paracellular water flow.

MATERIALS AND METHODS

Tissues and Solutions

The techniques used to isolate and mount the gallbladder were in general as described in previous publications (Altenberg, Copello, Cotton, Dawson, Segal, Wehner, and Reuss, 1990) and in the preceding paper (Reuss et al., 1992).

The control bathing solution (NaCl-Ringer's solution) had the same composition given in the preceding paper (mM): 90 NaCl, 10 NaHCO₃, 2.5 KCl, 1.8 CaCl₂, 1.0 MgCl₂, and 0.5 NaH₂PO₄, and was equilibrated with 1% CO₂/99% air. The pH was ~7.65 and the osmolality ~200 mosmol/kg. In most experiments, changes in osmolality were obtained by sucrose addition to or removal from a solution in which NaCl had previously been partially replaced with the sugar, keeping the osmolality equal to that of the control Ringer's solution. In some experiments, the change in osmolality was produced by adding NaCl to the Ringer's solution. In experiments in which changes in osmolality were produced by changing solution sucrose concentration, tetrabutylammonium chloride (TBACl) was added to all solutions at concentrations proportional to [sucrose] in order to estimate the time course of the sugar concentration changes at the cell surface (see below).

Electrical Measurements

Transepithelial (V_m) and cell membrane voltages (apical, V_{mc} ; basolateral, V_{cs}) were measured as described in the preceding paper (Reuss et al., 1992; see also Altenberg et al., 1990). In most cases, membrane voltages were measured with the electrolyte-filled barrel of double-barrel glass microelectrodes in which the other barrel was TMA⁺ sensitive (see below). Although most voltage traces were obtained in the absence of transepithelial current pulses to facilitate curve fitting procedures, current pulses were routinely applied shortly after cell penetrations to validate the impalements (Altenberg et al., 1990).

Electrical data were amplified, digitized at a rate of 10–100 Hz, and stored for later analysis with a laboratory computer.

Ion-selective Microelectrodes

A modified K⁺-selective microelectrode (Cotton and Reuss, 1989) referred to the apical solution voltage electrode was used to assess the time course of the change in TBA⁺ concentration at the cell surface in experiments in which Na⁺ was replaced with TBA⁺; the Na⁺ concentration change was calculated assuming that at the epithelial surface [Na⁺] + [TBA⁺] is constant. Similar measurements were used to estimate the time course of changes in sucrose concentration at the epithelial surface when sucrose was added to or removed from the bathing medium. To this end, TBA⁺ was added to the control NaCl-Ringer's solution and to the sucrose-containing solutions at different concentrations, and the fractional change in [sucrose] was assumed to be equal to the fractional change in [TBA⁺] (Cotton, Weinstein, and Reuss, 1989).

Double-barrel, intracellular, TMA⁺-sensitive microelectrodes were constructed and calibrated as described before (Reuss, 1985; Cotton et al., 1989). They were used to measure changes in intracellular [TMA⁺] upon osmotic challenge in cells preloaded with TMA⁺ by the nystatin technique (Reuss, 1985; Cotton et al., 1989). The difference between the voltage output of the TMA⁺-sensitive barrel and the reference barrel yields data from which the intracellular [TMA⁺] can be calculated. The intracellular [TMA⁺] remains unchanged for as long as 3 h (Reuss, 1985), indicating that after nystatin removal the cell membranes are impermeable to TMA⁺. Hence, changes in the differential voltage signal denote changes in the intracellular effective osmolality.

Data Analysis

Results are given as means \pm standard error; statistical comparisons were made by Student's *t* test applied to paired data. A value of $P < 0.05$ was considered significant.

Curve Fitting

Nonlinear, least-squares curve fitting of the solution to the differential equation for the change in transepithelial potential to the measured potential change (see Model) was accomplished using MLAB (Civilized Software, Bethesda, MD), a general mathematical modeling package.

MODEL

After the addition of sucrose to the apical bathing solution water will flow from the cells into the apical bathing solution, i.e., across the apical cell membrane. This will cause an elevation in cell osmolality (Cotton et al., 1989). Because of the high osmotic water permeability of the lateral cell membranes (Persson and Spring, 1982; Cotton et al., 1989), the rise in intracellular osmolality will cause a rapid water flux from the lateral intercellular spaces into the cells, thereby resulting in an increase in solute (NaCl) concentration in the spaces. If the junctions have a finite osmotic water permeability, transjunctional water flux from the lateral intercellular spaces to the apical bathing solution will contribute to the increase in [NaCl] in the spaces. Because of the apical unstirred layer, water flow will also tend to reduce the [NaCl] at the apical surface of the epithelium by a sweeping-away effect.

Inasmuch as the junctions are cation selective, i.e., $P_{Na} > P_{Cl}$ (Van Os and Slegers, 1975; Reuss and Finn, 1975), the transjunctional NaCl concentration difference will cause a lumen-positive change in V_m . The following is a derivation of the time course of the change in V_m after exposure of the apical cell surface to a hyperosmotic solution by addition of an impermeant nonelectrolyte.

Consider a layer of cells of volume V , with nominal apical surface area = 1 cm^2 . The cells contain effectively impermeant solutes at a total concentration C . The lateral intercellular spaces are assumed to be at osmotic equilibrium with the cells (Cotton et al., 1989), and the increase in apical solution osmolality causes the entire epithelial slab (cells and lateral intercellular spaces) to shrink uniformly. We also assume that concentration gradients within the cell are negligible. The initial concentration of both apical and basolateral solutions is C_1 .

If initially $C = C_1$ and $V = V_1$, then

$$C_1 \cdot V_1 = C(t) \cdot V(t) \quad (1)$$

where $C(t)$ is the concentration of C and $V(t)$ is the volume of the cell layer as functions of time. From the flux equation:

$$dV/dt = L_a \cdot [C(t) - C_2(t)] + L_b \cdot [C(t) - C_1] \quad (2)$$

where $C_2(t)$ is the osmolality at the apical surface, which is a function of time ($C_2(0) = C_1$). L_a and L_b are the effective hydraulic permeability coefficients of the

apical and basolateral barriers, respectively. Differentiating Eq. 1 and solving for dV/dt :

$$dV/dt = \frac{-C_1 \cdot V_1 \cdot (dC/dt)}{C^2(t)} \quad (3)$$

Substituting Eq. 3 into Eq. 2 and solving for dC/dt :

$$dC/dt = \frac{-C^2(t) \cdot \{L_a \cdot [C(t) - C_2(t)] + L_b \cdot (C(t) - C_1)\}}{C_1 \cdot V_1} \quad (4)$$

However, the change in osmolality at the apical surface (from C_1 to C_2) is not instantaneous, but can be approximated by a single exponential:

$$C_2(t) = C_2 + (C_1 - C_2) \cdot e^{-t/\tau} \quad (5)$$

where τ is the time constant of the change in osmolality at the epithelial surface. Substituting Eq. 5 into Eq. 4 we get

$$dC/dt = \frac{-C^2(t) \cdot \{L_a \cdot [C(t) - [C_2 + (C_1 - C_2) \cdot e^{-t/\tau}]] + L_b \cdot [C(t) - C_1]\}}{C_1 \cdot V_1} \quad (6)$$

The change in V_{ms} , assuming equilibration with the cell concentration, $C(t)$, is given by

$$\Delta V_{ms} = A(t) \cdot \log [C(t)/C_1] \quad (7)$$

where $A(t)$ depends on the junctional selectivity ($A(t) = 58.7$ mV for a junction perfectly selective for Na^+ at 24°C).

In the steady state, from Eqs. 6 and 7:

$$\Delta V_{ms} = A \cdot \log \left[\frac{L_a \cdot C_2 + L_b \cdot C_1}{(L_a + L_b) \cdot C_1} \right] \quad (8)$$

In the native *Necturus* gallbladder epithelium, the cell volume changes elicited by changes in apical solution osmolality are close to those predicted for an osmometric response (Persson and Spring, 1982; Cotton et al., 1989), indicating that the effective hydraulic resistance of the barriers on the basolateral side of the tissue are much higher than those on the apical side. This is undoubtedly due to the presence of subepithelial tissue layers, since in isolated proximal renal tubules the diffusion resistance of the lateral intercellular spaces is very low (Schafer, Patlak, and Andreoli, 1975, 1977). If effective $L_a \gg$ effective L_b ,¹ then

$$\Delta V_{ms} = A \cdot \log \frac{C_2}{C_1} \quad (9)$$

Three models for the time course of the junctional selectivity were considered. In the first, the selectivity was assumed to change to a final value at the instant of the solution change so that $A(t)$ in Eq. 7 is constant and equal to A_2 , the steady-state

¹ The use of a model including a finite hydraulic permeability of the basolateral barriers yielded a good fit to the data for an effective basolateral L_p about one-fourth of the apical one. As shown before (Cotton et al., 1989), this is consistent with changes in salt concentration on the basolateral regions of the epithelium and does not imply a lower L_p of the basolateral cell membrane.

selectivity at the ionic strength of the final solution. In the second and third models, $A(t)$ was allowed to vary from its initial value A_1 to its final value A_2 as a function of C or C^{-1} , i.e., according to the p th power of the concentration of the impermeant solute (where $p = 1$ or -1).² Thus:

$$A(t) = \frac{A_1 \cdot [C_2^p - C(t)^p] + A_2 \cdot [C(t)^p - C_1^p]}{C_2^p - C_1^p} \quad (10)$$

For the model calculations, Eqs. 6–8 were solved simultaneously, varying A_2 , L_a , and L_b to give the least-squares fit of Eq. 7 to the measured change in V_{ms} . The value of τ was determined separately by fitting a single exponential to the time course of the change in osmolality at the cell surface, assessed from the voltage output of an ion-sensitive microelectrode, using TBA⁺ as a sucrose tracer (see Materials and Methods).

RESULTS

In the preceding paper we compared the time courses of the transepithelial voltage changes produced by an apical isoosmotic partial substitution of NaCl with TBACl (bi-ionic potential), or by addition of sucrose to the NaCl-Ringer's apical bathing solution (apparent streaming potential). The half-times for the on and off voltage changes were longer for the apparent streaming potential, by 2.5- to 4-fold, and by 5- to 6-fold, respectively (Table V of Reuss et al., 1992). This result appears to be inconsistent with a true streaming potential, since in such a case the ion flow in response to the osmotic challenge should be linear with the difference in osmolality, and hence the streaming potential should develop *pari-passu* with the change in [sucrose] at the cell surface. That this is not the case is illustrated in Fig. 1, where the time courses of the changes in V_{ms} elicited by adding or removing sucrose from the apical bathing solution are compared with the time courses of the changes in sucrose concentration at the apical surface, estimated from V_{TBA} . The bi-ionic change in V_{ms} is included for comparison and compared with the rate of change of $-\log(Na_i/Na_o)$ at the epithelial surface. It is our contention (see Discussion) that the Na⁺/TBA⁺ junctional bi-ionic potential should have virtually the same time course as a true streaming potential produced by sucrose addition if they originate at the same site. In addition, for a true streaming potential the time course of ΔV_{ms} should be linear with the osmolality, and hence [sucrose]. The experimental findings are that the onset of the apparent streaming potential is much slower than that of the bi-ionic potential. Furthermore, the relationship between V_{ms} and the sucrose concentration at the cell surface is clearly nonlinear. In contrast, the time courses of V_{ms} and $-\log(Na_i/Na_o)$ are very close, as expected for a simple diffusion potential. The small difference in time courses is likely to result from a more rapid fluid exchange at the site of measurement of the [TBA⁺]; the change in V_{ms} is slower because it reflects solution mixing throughout the apical surface of the epithelial sheet. These observa-

² An inverse relationship between junctional ion selectivity and lateral intercellular space NaCl concentration was first considered because of the possibility that the change in selectivity could result from screening of fixed charges by changes in ionic strength. Experiments designed to test this possibility yielded negative results (see below).

tions indicate that either the voltage change elicited by sucrose addition is not a true streaming potential, or it does not originate at the same site as the bi-ionic potential (for instance, sucrose must diffuse to a deeper site in the junction).

Time Courses of Apparent Streaming Potentials, Sucrose Concentration at the Cell Surface, and Cell Water Volume

We first consider the hypothesis that the apparent streaming potentials are really pseudo-streaming potentials. In the case of addition of sucrose to the apical bathing

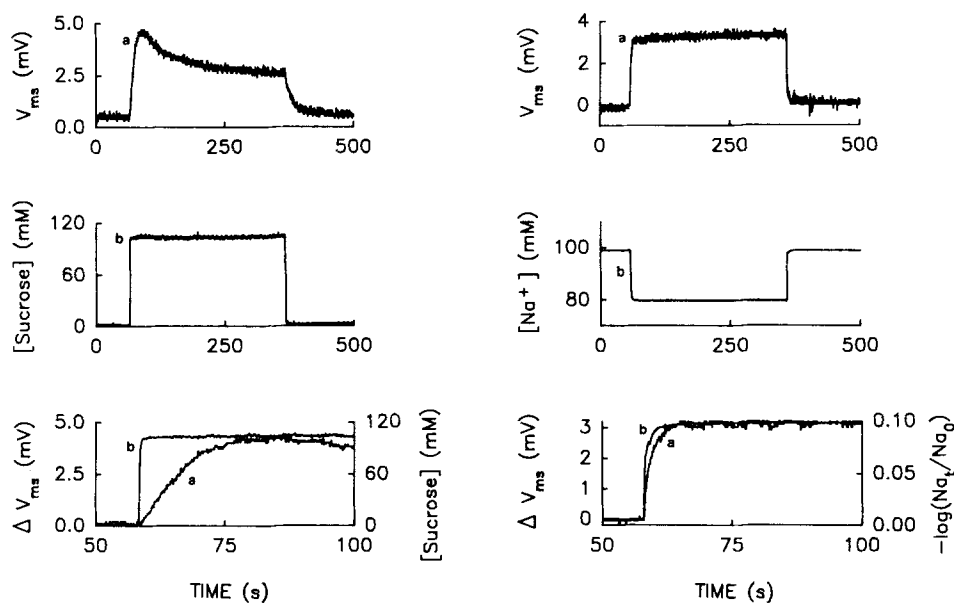


FIGURE 1. Top records: time courses of transepithelial voltage changes (V_{ms}) elicited by addition of sucrose (100 mM) to the apical bathing solution (*left*) and by isomolar substitution of 20 mM Na^+ with 20 mM TBA⁺ (*right*). Middle records: time courses of [sucrose] and $[Na^+]$ at the cell surface, calculated from the difference between the voltage output of a TBA⁺-sensitive microelectrode positioned near the apical surface of the epithelium and V_{ms} . Bottom records: plots of V_{ms} and [sucrose] vs. time and of V_{ms} and $-\log [Na_i/Na_o]$ vs. time, respectively. For a true streaming potential elicited by sucrose addition, and for a paracellular bi-ionic potential by partial Na^+ substitution, the relationships should be linear, i.e., the scaled time courses should overlap. Note that the prediction holds for the bi-ionic, but not for the apparent streaming potential (see text).

solution, the water flow from the cell causes an elevation in cell osmolality, which in turn elicits osmotic water flow from the lateral intercellular spaces to the cells. The final result will be an increase in $[NaCl]$ in the fluid contained in the spaces. Inasmuch as the junctions are cation selective ($P_{Na} > P_{Cl}$), the transjunctional salt concentration gradient causes a lumen-positive change in V_{ms} . Therefore, the change in lateral space $[NaCl]$ must "follow" the change in concentration of the cell water

volume marker (TMA^+). We tested this prediction by comparing the time courses of V_{ms} , V_{TMA} (cell water volume signal), and V_{TBA} (apical solution osmolality signal) in response to an increase in apical solution osmolality produced by sucrose addition. The result is shown in Fig. 2. *A* shows the time courses of the three voltages. The plot in *B* compares the values of V_{ms} and V_{TBA} at all times of the records depicted in *A* (trajectory denoted by the sequence *a-b-c-d*). There is a clear dissociation of the two time courses: the change in V_{TBA} (*a-b*) is virtually complete at the time that the V_{ms} change starts (*b*); upon sucrose removal, the change in V_{TBA} (*c-d*) also precedes the change in V_{ms} (*d-a*). In plot *C*, we compare the time courses of V_{TMA} and V_{ms} . In contrast with the result depicted in *B*, the data are well described by a straight line,

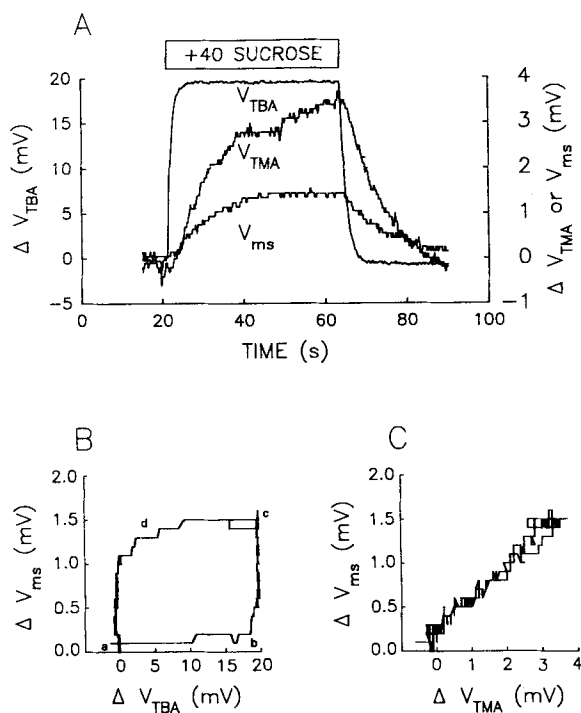


FIGURE 2. (*A*) Effects of addition of 40 mM sucrose to the apical bathing solution (*bar*) on V_{ms} , V_{TMA} , and V_{TBA} . V_{TMA} and V_{TBA} are the intracellular TMA^+ and extracellular TBA^+ signals, respectively, recorded with ion-selective microelectrodes as described in Materials and Methods. (*B*) Comparison of the changes in V_{ms} and V_{TBA} . The trajectory is *a-b-c-d*. Upon sucrose addition, the change in V_{TBA} is complete (*a-b*) before the change in V_{ms} (*b-c*); upon sucrose removal, again the change in V_{TBA} is essentially complete (*c-d*) before the change in V_{ms} (*d-a*). (*C*) Comparison of the changes of V_{ms} and V_{TMA} . Note that V_{ms} is linearly related to V_{TMA} .

with a slight hysteresis at times ranging from 45 to 60 s. Similar results were obtained in experiments on five tissues. The main parameters are summarized in Table I. These results are consistent with the hypothesis proposed above, namely, that the change in V_{ms} is a pseudo-streaming potential whose time course follows the change in cell volume.

Fig. 3 shows the result of an experiment in which sucrose was removed from the apical bathing solution. All voltage changes are opposite in polarity to those shown in Fig. 2, as expected, and the same time correlations hold: V_{ms} is delayed with respect to V_{TBA} and follows closely V_{TMA} . Results obtained with sucrose removal in four tissues are summarized in Table I.

TABLE I
Half-Times of the Changes in V_{ms} , V_{TBA} , and V_{TMA} Elicited by Increases or Decreases in Apical Solution Sucrose Concentration

$\Delta[\text{Sucrose}]$	Half-times		
	ΔV_{ms}	ΔV_{TBA}	ΔV_{TMA}
mM			
+40	11.2 ± 1.5	$2.0 \pm 0.6^*$	9.0 ± 2.0
-40	10.4 ± 2.6	$2.7 \pm 1.2^*$	11.0 ± 3.3

Data are means \pm SEM of $n = 5$ and $n = 4$ experiments, for +40 and -40 mM, respectively. Protocols as in Figs. 2 and 3. The apical solution sucrose concentration was changed as indicated in the first column, keeping the ion concentrations constant.

*Significantly different from half-time for ΔV_{ms} ($P < 0.05$).

Fits to Model

The model described above should predict accurately the time course of V_{ms} and yield data on the osmotic permeability coefficients of the apical barrier (L_p) and the junctional Na^+/Cl^- selectivity. Fig. 4 (left) shows an example of a fit of the model equations (assuming a change in junctional selectivity proportional to $1/C_j$, where C_j is the $[\text{NaCl}]$ in the lateral intercellular spaces) to the initial 30 s of the V_{ms} change elicited by adding 50 mM sucrose to the apical bathing solution. The plot on the right in Fig. 4 shows the analogous fit to the V_{ms} change elicited in the same preparation by 150 mM sucrose. The fits yield virtually the same results for the free

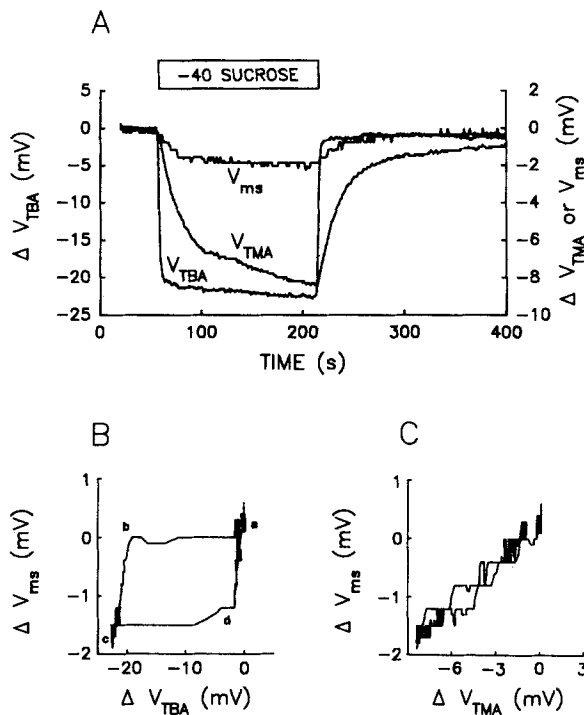


FIGURE 3. Effects of removal of 40 mM sucrose from the apical bathing solution (bar) on V_{ms} , V_{TMA} , and V_{TBA} . Format as in Fig. 2. Again the changes in V_{ms} and V_{TMA} are linearly related, but the change in V_{ms} differs from that of V_{TBA} .

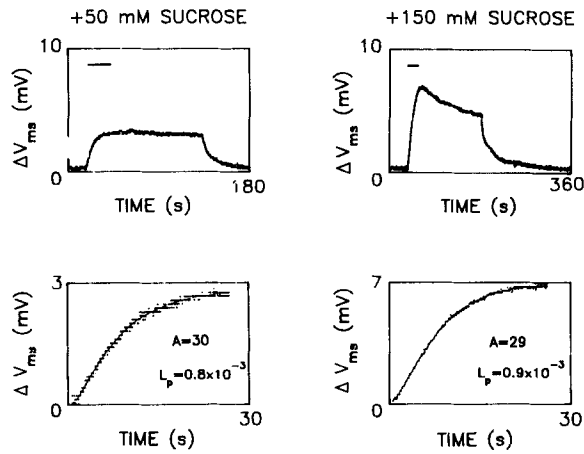


FIGURE 4. Model fits of the V_{ms} changes elicited by increasing apical solution osmolality by addition of sucrose at the concentrations indicated. Upper panels show V_{ms} values for 3 and 6 min, respectively. The lower panels depict data for the periods indicated by the lines on the top of the records in the upper panels, and fitted curves superimposed on the experimental data. Note that the fitted parameters (A in mV, L_p in $\text{cm}\cdot\text{s}^{-1}\cdot(\text{osmol}/\text{kg})^{-1}\cdot 10^{-3}$) are virtually the same for the two osmotic challenges.

parameters: L_p and A , which correspond to the hydraulic permeability coefficient of the apical barrier and the apparent junctional selectivity (see Model), respectively.

In Fig. 5, the model equations were fit to the V_{ms} change elicited by addition of sucrose to the apical solution to a final nominal concentration of 50 mM, and the parameters were then used to generate the curves superimposed with the V_{ms} changes

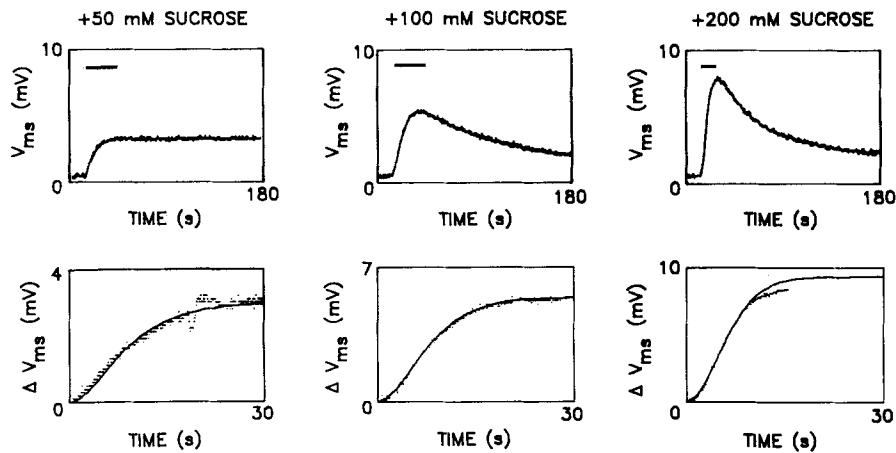


FIGURE 5. Protocol as in Fig. 4. Top panels depict V_{ms} data and bottom panels show data for the periods indicated by the lines on the top of the records in the upper panels, and fitted curves superimposed on the experimental data. The panels on the left depict an experiment in which the apical solution osmolality was increased by addition of sucrose to a final nominal concentration of 50 mM. The fitted parameters ($A = 28$ mV, $L_p = 1.5 \cdot 10^{-3} \text{ cm}\cdot\text{s}^{-1}\cdot[\text{osmol}/\text{kg}]^{-1}$) were used to generate the curves superimposing the data in experiments in which the apical surface of the epithelium was exposed to 100 and 200 mM sucrose (second and third panels, respectively). Note that for 200 mM sucrose the fitted curve describes the initial voltage changes but deviates later on.

produced by 100 and 200 mM sucrose (lower panels). Note that the fits are excellent for the initial segments, and deviate later on. The deviation is probably caused by the slow increase in paracellular electrical resistance elicited by large osmotic gradients (see Discussion).

Table II summarizes the results of model fits in several tissues, for addition of 100 mM sucrose. The average L_p values were 1.2×10^{-3} and 0.8×10^{-3} $\text{cm}\cdot\text{s}^{-1}\cdot(\text{osmol}/\text{kg})^{-1}$, for the model assuming instantaneous and slow changes in junctional selectivity, respectively. Both values are in good agreement with that obtained directly from cell water volume measurements (0.8×10^{-3} $\text{cm}\cdot\text{s}^{-1}\cdot(\text{osmol}/\text{kg})^{-1}$; Cotton et al., 1989). The simplest model, i.e., assuming an instantaneous change in junctional selectivity, results in an overestimate of L_p . The value of A from both fits depends on the pseudo steady-state change in V_{ms} , and hence is the same for both models.

The model calculations indicate a relatively low junctional selectivity during the apparent streaming potential. For instance, if the [NaCl] in the lateral intercellular spaces reaches 150 mM in a 100 mM sucrose experiment, using the junctional

TABLE II
Fitted Model Parameters (A = Junctional Selectivity, L_p = Apical Membrane Hydraulic Permeability Coefficient) for a 100-mM Addition of Sucrose to the Apical Bathing Solution

Model	A	L_p
	<i>mV</i>	$\text{cm}\cdot\text{s}^{-1}\cdot(\text{osmol}/\text{kg})^{-1}\cdot 10^{-3}$
Step change	19.4 ± 1.8	1.21 ± 0.23
$A = f(1/C_i)$	19.1 ± 2.1	$0.76 \pm 0.18^*$

Data are means \pm SEM of $n = 10$ experiments. Model fits were carried out assuming an instantaneous change in selectivity upon increasing apical solution sucrose concentration (top line) or assuming that A changes continuously in proportion to $1/C_i$ (bottom line), where C_i is the salt concentration in the lateral intercellular space (see Model).

*Significantly different from value calculated for a step-change model.

selectivity estimated from elevations of apical bathing solution [NaCl], the change in V_{ms} at constant selectivity should be ~ 5.3 mV, which is consistently greater than the observed change. Clearly, the magnitude of the change in transepithelial voltage upon addition of sucrose to the apical bathing solution depends on the change in lateral intercellular space [NaCl] and in the junctional Na^+/Cl^- selectivity. It is possible that our calculations underestimate the selectivity because of an overestimate of the increase in [NaCl] in the lateral intercellular spaces. To quantify directly the junctional selectivity changes during the apparent streaming potential, we carried out 20-s substitutions of 20 mM NaCl with 20 mM TBACl before and during the voltage change elicited by addition of 100 mM sucrose. As shown in Fig. 6, the bi-ionic change in V_{ms} during the apparent streaming potential was reduced significantly compared with control values before or after the hyperosmotic challenge. The effect was virtually complete 20 s after the addition of sucrose. Table III shows a comparison between the junctional selectivity parameter A measured under control conditions, calculated from model fits, and measured during the apparent streaming

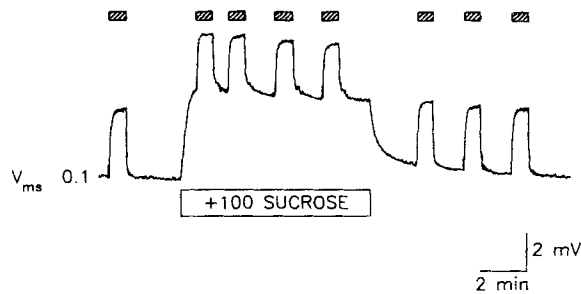


FIGURE 6. Comparison of the V_{ms} changes produced by isomolar replacement of 20 mM NaCl with TBACl (bi-ionic potentials, top bars) under control conditions, during addition of 100 mM sucrose to the apical bathing solution, and after sucrose removal. Note the reversible reduction in the bi-ionic potentials during the apparent streaming potential, which is due to a decrease in junctional ion selectivity (see text).

potential. The values of A were calculated from the bi-ionic potentials comparing the expected Nernstian response for a perfectly Na^+ selective junction and the observed change in V_{ms} , according to:

$$A = 58.7 \cdot (\Delta V_{ms} / \Delta V^*) \quad (11)$$

where $\Delta V^* = -58.7 \cdot \log(80/100)$, i.e., the Nernstian voltage change. As shown in Table III, the changes in selectivity estimated from the model fits are in excellent agreement with the direct measurement.

The preceding analysis is based on the assumption that the V_{ms} change produced by the TBA^+/Na^+ substitution is entirely paracellular in origin. To rule out the contribution of cell membrane parameters, we carried out the intracellular microelectrode experiments summarized in Table IV. The changes in fR_a and in cell membrane voltages are too small to explain the change in V_{ms} . For instance, assuming $R_{bl} = 1,000 \Omega \cdot \text{cm}^2$ (Stoddard and Reuss, 1988), a 6-mV depolarization of the basolateral membrane zero-current voltage (see Table IV) would cause a V_{ms} change of $\sim 6 \cdot (200/5,200) = 0.2$ mV, where $R_s = 200$ and $(R_a + R_{bl} + R_s) = 5,200 \Omega \cdot \text{cm}^2$ (see Eq. 4 in Reuss et al., 1992). Taken together, these results indicate that the NaCl/TBACl bi-ionic potential is of paracellular origin and that the junctional selectivity is reduced rapidly by apical solution hyperosmolality. In the next section

TABLE III
Junctional Selectivity (A , mV) under Control Conditions and upon Addition of 100 mM Sucrose to the Apical Bathing Solution

Control	Step change	$A = f(C)$	Measured
34.8 ± 4.1	$22.7 \pm 1.7^*$	$22.9 \pm 1.7^*$	$22.8 \pm 2.8^*$

Data are means \pm SEM of $n = 4$ experiments. Column 1: A in isoosmotic Ringer's solution, measured from the change in V_{ms} elicited by substitution of 20 mM NaCl with 20 mM TBACl in the apical bathing solution. Columns 2 and 3: A obtained according to the models described in Table II (see Model). Column 4: A calculated from the V_{ms} change produced by a 20-mM TBACl/NaCl substitution at 1 min of exposure to the hyperosmotic apical bathing solution (see Figs. 6 and 9).

*Significantly different from control value.

we present experimental results that indicate that the change in selectivity involves a reduction of junctional $P_{\text{Na}}/P_{\text{Cl}}$.

In addition to the rapid changes in junctional selectivity presented here, changes in transepithelial resistance during exposure to sucrose elicit late V_{ms} transients, as shown in the preceding paper (Reuss et al., 1992).

Simultaneous Generation of Apparent Streaming Potentials and Diffusion Potentials by NaCl Addition to the Apical Bathing Solution

The slow early time course of the change in V_{ms} produced by addition of sucrose to the apical bathing solution supports the hypothesis of a pseudo-streaming potential. Further, this conclusion is quantitatively strengthened by the comparison of the time courses of V_{ms} and intracellular V_{TMA} upon hyper- and hypoosmotic challenges. However, the difference between the time courses of bi-ionic and apparent streaming potentials could have an alternative explanation, i.e., the sites at which the effects of Na^+ substitution and sucrose addition occur could be different. For instance, sucrose

TABLE IV
Changes in Cell Membrane and Transepithelial Electrophysiologic Parameters Elicited by Replacing 20 mM NaCl with 20 mM TBACl in the Apical Bathing Solution

Condition	V_{m}	V_{mc}	V_{cs}	fR_{e}	R_{t}
	mV	mV	mV		$\Omega \cdot \text{cm}^2$
Control	-0.3 ± 0.3	-69 ± 3	-69 ± 4	0.87 ± 0.03	196 ± 16
TBACl, 1 min	$3.1 \pm 0.2^*$	-66 ± 4	$-63 \pm 4^*$	0.81 ± 0.02	$221 \pm 20^*$
TBACl, 2 min	$3.0 \pm 0.2^*$	$-63 \pm 3^*$	$-60 \pm 3^*$	$0.76 \pm 0.03^*$	$224 \pm 21^*$
Control	-0.3 ± 0.3	-66 ± 3	-66 ± 3	0.82 ± 0.04	200 ± 21

Data are means \pm SEM of $n = 7$ experiments. The four lines denote control values (NaCl-Ringer's solution), experimental values at 1 and 2 min (apical solution substitution of 20 mM NaCl with 20 mM TBACl), and control values 3 min after returning to NaCl-Ringer's solution.

*Significantly different from control ($P < 0.05$).

might have to reach a deeper site in the junction to elicit the water flow ultimately responsible for the change in transepithelial voltage. To test this possibility, we carried out experiments designed to induce both a paracellular diffusion potential and an apparent streaming potential by addition of a single solute to the apical bathing solution. Taking advantage of the high value of junctional $P_{\text{Na}}/P_{\text{Cl}}$, we added NaCl to the control apical Ringer's solution. The expectation is that V_{ms} should change rapidly in the mucosa-negative direction because of a diffusion potential due to the elevated apical solution [NaCl]. Later on, the voltage should return toward the control value, i.e., the change in V_{ms} should be in the mucosa-positive direction, as the [NaCl] in the lateral intercellular spaces rises because of the water flux from the spaces to the cells and to the lumen. Furthermore, the time course of the second (slow) voltage change should be similar to that produced by sucrose addition to a similar final osmolality. The result of the experiment is shown in Fig. 7. The two predicted time courses are clearly observed, and the slow mucosa-positive V_{ms} change follows a similar time course to that produced by sucrose addition. Similar results

were obtained in four experiments. This result rules out the possibility that the differences in time course between Na^+/TBA^+ bi-ionic potentials and sucrose-induced apparent streaming potentials can be explained by the different properties of the solutes.

These experiments with NaCl addition to the apical bathing solution allowed us to assess the change in junctional selectivity by comparing the on and off voltage changes upon transient exposure to the high-NaCl solution. Fig. 8 shows the results

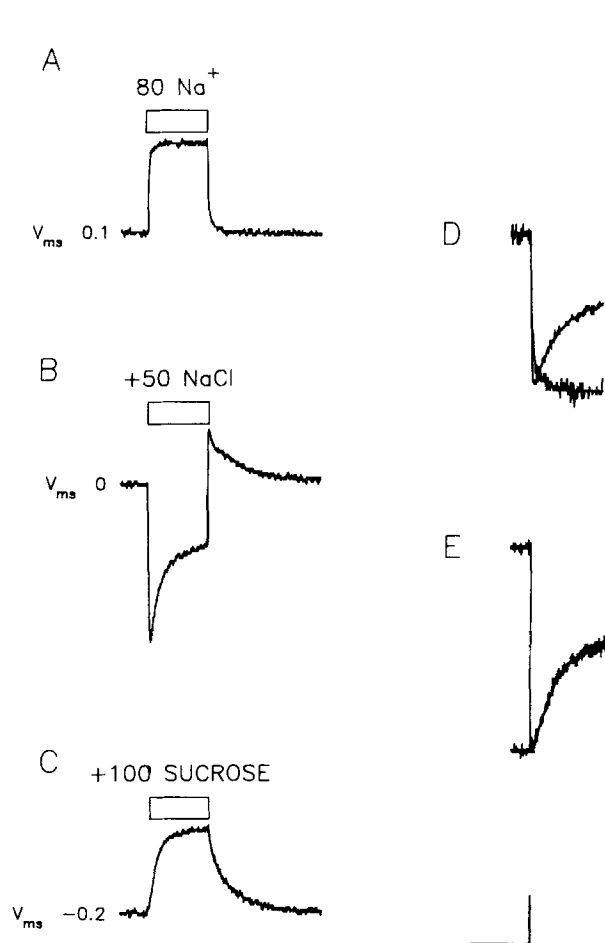


FIGURE 7. (A) V_{ms} change produced by a reduction of $[\text{Na}^+]$ from 100 to 80 mM (isomolar replacement of 20 mM Na^+ with TBA^+) in the apical bathing solution. (B) V_{ms} change produced by addition of 50 mM NaCl to the apical bathing solution. (C) V_{ms} change produced by addition of 100 mM sucrose to the apical bathing solution. All traces were obtained in the same preparation. (D) Superimposition of the initial part of the trace in B and the inverted (scaled) trace in A. (E) Superimposition of the trace in B (starting at the peak negative voltage) and the trace in C (starting at the onset of the voltage change). Scales: 2 mV, 1 min for records A, B, and C. In D and E, the time calibration is 24 s; in D the inverted trace was scaled. The V_{ms} change in response to NaCl addition has two distinct phases: a rapid one that has a similar time course to the Na^+/TBA^+ bi-ionic potential and a slow one that has a similar time course to that of the apparent streaming potential produced by sucrose addition.

of a representative experiment in which the exposure to high $[\text{NaCl}]$ was varied between 15 s and 3 min. The off response was reduced when the exposure to the hyperosmotic solution was longer, indicating that the junctional Na^+/Cl^- selectivity falls as a function of time. The selectivity was estimated for each off response, according to

$$\Delta V_{ms} = A \ln (150/100) \quad (12)$$

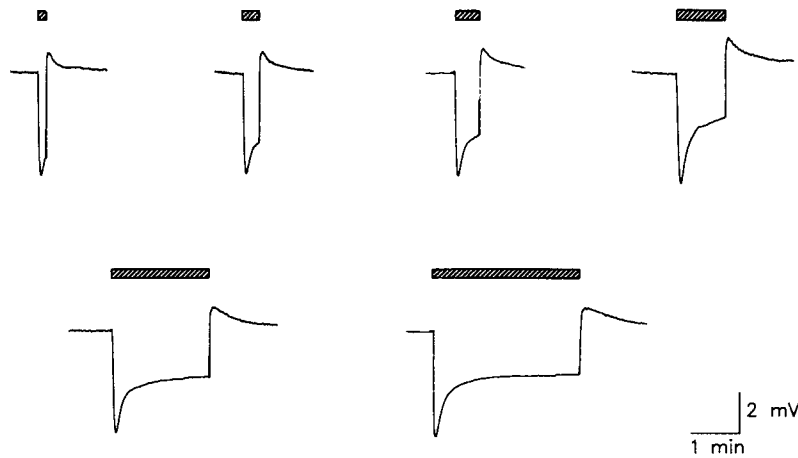


FIGURE 8. V_{ms} changes produced by transient addition of NaCl (50 mM) to the apical bathing solution, for periods varying from 15 s to 3 min (left to right). The V_{ms} changes upon returning to control Ringer's solution on the apical side were used to calculate the junctional Na^+/Cl^- selectivity as described in the text.

where $A = (RT/F)[(P_{\text{Na}} - P_{\text{Cl}})/(P_{\text{Na}} + P_{\text{Cl}})]$, P denotes junctional permeability, and 150 and 100 represent the two $[\text{NaCl}]$ in the apical bathing solution. Eq. 12 is valid for a rapid solution change, such that the $[\text{NaCl}]$ in the lateral intercellular space and its electrical resistance are the same at the time immediately preceding the solution substitution and the peak change in V_{ms} . The average time course of A is compared in

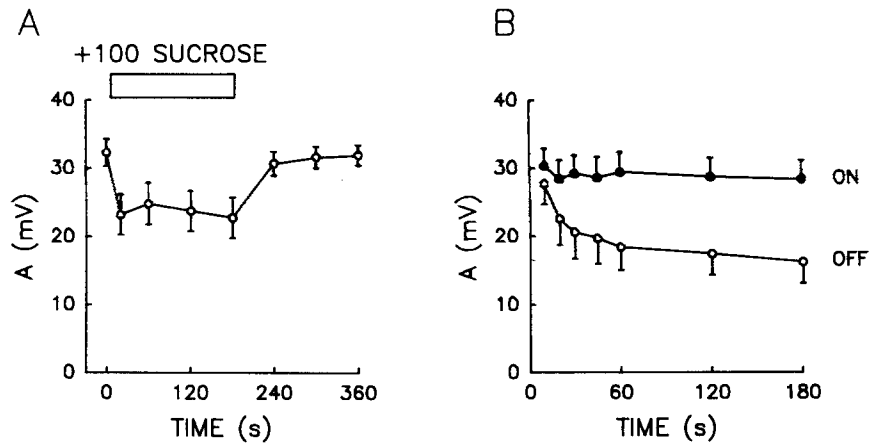


FIGURE 9. (A) The apparent junctional selectivity (A , mV), defined by Eqs. 11 and 12, was calculated from bi-ionic potentials before, during, and after addition of 100 mM sucrose to the apical bathing solution (see Fig. 6); $n = 4$ experiments. The selectivity falls reversibly during apical exposure to 100 mM sucrose. (B) The apparent junctional selectivity (A , mV) was calculated from the on (●) and off (○) responses to a transient exposure to 150 mM NaCl on the apical side, for the time denoted by the abscissa (see Fig. 8); $n = 4$ experiments. The selectivity calculated from the off responses falls as a function of duration of the elevation in apical solution $[\text{NaCl}]$ (see text).

Fig. 9 with the analogous parameter for the experiments in which Na^+ was partially replaced with TBA^+ before, during, and after addition of 100 mM sucrose to the apical bathing solution (Fig. 6). The parameter A in the bi-ionic potential experiments was estimated from a comparison of the expected Nernstian response for a perfectly Na^+ selective junction and the observed change in V_{ms} , as described above.

The similar time courses of the reductions of A in the two sets of experiments support the hypothesis that the elevation of apical solution osmolality causes a rapid fall in junctional selectivity, i.e., in $P_{\text{Na}}/P_{\text{Cl}}$. The concomitant elevation of R , suggests a decrease in P_{Na} , but this conclusion is uncertain because of the possible change in the series resistance of the lateral intercellular spaces.

DISCUSSION

The Time Course of a True Streaming Potential Should Be Linear with the Change in Bathing Solution Osmolality

A central argument in the interpretation of our results is that a "true" junctional streaming potential should have a time course linear with the change in osmolality in the apical bathing solution in the immediate vicinity of the junctions (House, 1974). In fact, streaming potential models based on slits or pores indicate such a linearity. In the analysis of Katchalsky and Curran (1965), the zero-current streaming potential (ΔE) is given by

$$\Delta E = -\beta \Delta p \quad (13)$$

where β is the electro-osmotic coefficient and Δp is the hydrostatic pressure difference.

In the case of an osmotic driving force ($\Delta p = 0$), Eq. 13 becomes $\Delta E = \beta \Delta \pi$.

In simple terms, a true streaming potential has no sizable latency or capacitative component vis-à-vis the osmolality (or hydrostatic pressure) change, because the voltage change is directly related to the water flux via a coupling coefficient which represents the frictional interaction between the water and ion flows. This view is supported by observations of electroosmotic phenomena in artificial membranes (Schmid and Schwarz, 1952), plant cells (Barry and Hope, 1969*a, b*), and animal cells, both nonpolar (Vargas, 1968; Stallworthy, 1970) and epithelial (House, 1964; Wedner and Diamond, 1969). When appropriately assessed, the "true" electroosmotic component was fast relative to events due to polarization (i.e., transport number) effects. The same problem, i.e., the experimental coexistence of "true" and "pseudo" streaming potentials, has been clearly identified in artificial and biological membranes (House, 1964, 1974; Schmid and Schwarz, 1952; Vargas, 1968; Wedner and Diamond, 1969). The main point is that the presence of unstirred fluid compartments complicates the interpretation of transmembrane voltage changes in response to hydrostatic or osmotic pressure gradients. This point has been eloquently discussed by House (1974), Diamond (1979), and Barry and Diamond (1984).

Our results rule out that the ΔV_{ms} elicited by addition of sucrose to the apical bathing solution is a true streaming potential because the time courses of the change in transepithelial voltage and surface solution osmolality differ widely, as best illustrated in Fig. 1. In addition, the rate of change of osmolality at the epithelial

surface cannot account for the difference, for two reasons: first, because it was assessed from the time course of the change in TBA⁺ concentration, which is a reasonable indication of the time course in sucrose concentration at the same site (Cotton et al., 1989), and second, because the comparison between the time courses of apparent streaming potentials and bi-ionic potentials reveals the same discrepancy. Assuming that the sites of generation of the bi-ionic potential and the apparent streaming potential are the same (namely, ion-selective sites in the junctions), our data unambiguously indicate that the observed voltage change is not a true junctional streaming potential.

Hence, we are left with the following alternative explanations: (a) the site at which sucrose addition elicits the voltage change is not the same as the Na⁺/TBA⁺ selectivity site, i.e., sucrose must reach a deeper site to elicit the voltage change, and this accounts for the slow development of ΔV_{ms} ; (b) ΔV_{ms} is not a true, but a pseudo-streaming potential (Diamond, 1979; Barry and Diamond, 1984), i.e., the slow time course is due to the change in [NaCl] in the lateral intercellular spaces, which occurs secondarily to the change in cell volume. In the next sections we discuss these possibilities in the light of our experimental results.

The Slow Time Course of the Apparent Streaming Potential Is Not Due to Slow Arrival of the Osmotically Active Solute to the Relevant Site

To rule out the possibility that sucrose exerts its effect on V_{ms} at a deeper junctional site than that responsible for the generation of bi-ionic potentials, we tested the effect of osmotic challenges produced by addition of NaCl to the apical bathing solution. If the apparent streaming potential is slow because it depends on cell shrinkage, and since the junctions are cation selective ($P_{Na} > P_{Cl}$), then addition of NaCl should result in a transepithelial voltage change with two distinct components: (a) a rapid negative ΔV_{ms} corresponding to the junctional diffusion potential caused by the difference in [NaCl] between apical bathing solution and lateral intercellular space fluid; and (b) a slow positive ΔV_{ms} due to the loss of water from the cells, followed by water loss from the spaces and an increase in lateral space [NaCl]. As shown in Fig. 8, the experimental observation is exactly as predicted by the pseudo-streaming potential hypothesis. If NaCl addition induced a true junctional streaming potential, one would expect the site to be the same as for a NaCl diffusion potential. Hence, on the basis of this result, we can reject the possibility of a "deep" site of action of the osmoticant.

The Time Course of the Apparent Streaming Potential Follows the Time Course of Cell Volume and Intracellular Osmolality

The essential observations in this work were summarized in Figs. 2 and 3. During sucrose addition to or removal from the apical bathing solution, the relationship between ΔV_{ms} and ΔV_{TMA} (using the intracellular [TMA⁺] as a measure of cell water volume and hence effective osmolality) was linear, whereas no such relationship was observed between ΔV_{ms} and ΔV_{TBA} (using the extracellular [TBA⁺] as a measure of [sucrose] at the epithelial surface). This result is the strongest argument for a pseudo-streaming potential.

Magnitude of the Apparent Streaming Potentials

The model calculations were based on the assumption of rapid osmotic equilibrium between the cells and the lateral intercellular spaces. Both optical and electrometric measurements of cell volume changes upon rapid alterations in apical solution osmolality in the native epithelium show that the volume changes are close to those predicted for an osmometric response (Persson and Spring, 1982; Cotton et al., 1989). Therefore, if upon apical solution osmolality changes the cell osmolality approaches that of the solution, and if the spaces equilibrate rapidly with the cells, then the steady-state salt concentration in the spaces could be easily calculated. For a Ringer's solution plus 100 mM sucrose, the steady-state [NaCl] in the spaces should be ~150 mM. From the junctional selectivity, estimated from diffusion potentials elicited by changes in apical solution [NaCl] (Figs. 7 and 9), we can estimate that such a gradient should result in a V_{ms} change of ~5.3 mV, but the observed changes in the same tissues were significantly less, on average 3.2 mV. The magnitude of the pseudo-streaming potential depends on the NaCl concentration ratio between lateral intercellular spaces and apical bathing solution, and on the junctional Na^+/Cl^- selectivity. Hence, the discrepancy between predicted and measured ΔV_{ms} could indicate that the model estimate of lateral intercellular space [NaCl] is incorrect, or that the junctional selectivity is changed by elevating the osmolality of the apical bathing solution.

Estimates of selectivity changes were obtained by 20-s partial substitutions of NaCl with TBACl before, during, and after addition of sucrose and by comparing the on and off V_{ms} changes upon transient elevations of [NaCl] in the apical bathing solution. As shown in Fig. 9, with both experimental protocols we observed a rapid decrease in junctional selectivity. To extract the selectivity from the changes in V_{ms} , we must assume that changes in cell membrane parameters do not contribute significantly to the V_{ms} changes produced by either the Na^+/TBA^+ substitution or the elevation of apical solution [NaCl]. In the case of bi-ionic potentials, the assumption is supported by measurements of cell membrane voltages and fR_a (Table IV), which revealed that changes in cell membrane parameters are insufficient to account for the measured ΔV_{ms} . For the transepithelial diffusion potentials elicited by transient elevations in apical solution [NaCl], we did not carry out concomitant measurements of cell membrane parameters. However, previous studies in this epithelium (see Reuss, 1989) indicate that the electrodiffusive Na^+ and Cl^- permeabilities of the apical membrane are low, and hence that the V_{ms} changes observed under these conditions are largely paracellular.

In conclusion, our results are consistent with the predictions for pseudo-streaming potentials and the model fits to the data account for the time course and the magnitude of the V_{ms} changes assuming that the lateral intercellular spaces equilibrate rapidly with the cells by osmotic water flow across the lateral cell membranes. Finally, our observations indicate that elevations in apical solution osmolality, by addition of either sucrose or NaCl, rapidly reduce the junctional Na^+/Cl^- selectivity.

Time Course of Osmotic Equilibration of the Lateral Intercellular Space

Comparison of the time courses of V_{TMA} and V_{ms} shows no measurable difference. If our conclusions pertaining to the mechanism of the V_{ms} change are correct, this

observation indicates that the lateral spaces reach rapid osmotic equilibrium with the cells. This conclusion is predictable from estimates of lateral space volume (Spring and Hope, 1978, 1979) and basolateral membrane L_p (Persson and Spring, 1982; Cotton et al., 1989). This is illustrated by the following calculation. Consider cells of height = 35 μm , with a cross-sectional area given by a regular hexagon of side = 20 μm , and consider the lateral intercellular spaces to be slits of constant width = 1 μm . The cell membrane L_p is $1.0 \times 10^{-3} \text{ cm}\cdot\text{s}^{-1}\cdot(\text{osmol}/\text{kg})^{-1}$. For this model, a step change in apical solution osmolality results in a change in cell water volume (and osmolarity) with a half-time of ~ 7.5 s; in contrast, a step change in cell osmolality results in a lateral space osmolality change with a half-time of ~ 100 ms. In other words, upon osmotic challenge the lateral spaces equilibrate rapidly with the cells; the effective delays are not measurable with presently available techniques.

Comparison with Results in Other Preparations

Electrokinetic and pseudo-electrokinetic phenomena have been demonstrated in many preparations of animal and plants cells (Diamond, 1962, 1966; Dietschy, 1964; House, 1964; Pidot and Diamond, 1964; Diamond and Harrison, 1966; Smyth and Wright, 1966; Clarkson, 1967; Vargas, 1968; Barry and Hope, 1969*a, b*; Stallworthy, 1970; Wright and Prather, 1970; Wright, Smulders, and Tormey, 1972; Rosenberg and Finkelstein, 1978; Alcayaga, Cecchi, Alvarez, and Latorre, 1989). In algae and in invertebrate giant axon two distinct time courses can be observed in response to rapid changes in hydrostatic or osmotic pressure (Vargas, 1968; Barry and Hope, 1969*b*; Stallworthy, 1970). However, the distinction between the two phenomena has been difficult in epithelial tissues.

Recent studies in epithelial preparations (e.g., Corman, 1985) suggest that the mechanism of apparent streaming potentials is in fact a polarization phenomenon such as that analyzed here, although the data do not allow for unambiguous resolution of the mechanism and site of origin of the observed voltage changes. A notable exception is the recent work of Tripathi and Boulpaep (1988). These authors measured apparent streaming potentials in *Ambystoma* proximal tubules perfused in vitro. From intracellular microelectrode experiments they concluded that the origin of the voltage changes upon changing perfusate or bath osmolality is paracellular. In this epithelium, the junctions are anion (i.e., Cl^-) selective. In contrast with our results with Na^+ removal, bilateral Cl^- removal did not reduce or reverse the apparent streaming potential. From this observation, Tripathi and Boulpaep (1988) concluded that their data were consistent with a true streaming potential of paracellular origin, mostly or exclusively along the lateral intercellular spaces. Two questions remain unanswered in their studies. First, the time course of the transepithelial voltage changes was slow relative to the stated rapidity of the solution changes (and also relative to the estimated changes in cell volume). Second, their observations would be consistent with a pseudo-streaming potential if the junctions are selective for cyclamate over Na^+ .

Conclusions

In summary, we have shown that the time course of the transepithelial voltage changes elicited across *Necturus* gallbladder epithelium by changing apical solution

sucrose concentration is inconsistent with a true streaming potential. The observation of a linear relationship between the transepithelial voltage change and the intracellular electrical signal produced by the change in concentration of a volume marker is a strong argument for a pseudo-streaming potential, i.e., a transepithelial voltage change dependent on the change in salt concentration in the lateral intercellular spaces. Further support for this conclusion was obtained in experiments in which a diffusion potential and an apparent streaming potential with distinct time courses were elicited by addition of only one solute, namely, NaCl, to the apical bathing solution.

Finally, our model calculations yield an L_p for the apical membrane that is in excellent agreement with the measured value (Cotton et al., 1989), and suggest that the junctional selectivity decreases rapidly upon increases in apical solution osmolality. This was directly confirmed by measuring bi-ionic potentials before, during, and after osmotic challenges. The mechanism of this reversible change in junctional selectivity is unknown.

We conclude that it is incorrect to ascribe voltage changes to a "true" streaming mechanism unless the time course of the voltage is proven to be linear with the change in osmolality at the relevant membrane surface. This is perhaps the best procedure currently available to examine this problem in epithelia. In *Necturus* gallbladder epithelium, our results rule out the existence of significant true streaming potentials, and hence argue against electrokinetic evidence for solute-solvent coupling in the paracellular pathway.

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